A CYTOLOGICAL STUDY OF BLEPHARISMA WITH SPECIAL REFERENCE TO ITS FIBRILLAR STRUCTURES

BY

CHARLES LEPLIE KANATZAR

B. Ed., Eastern Illinois State Teachers College, 1935

M. S., University of Illinois, 1936

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1940

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A CYTOLOGICAL STUDY OF BLEPHARISMA WITH SPECIAL REFERENCE TO ITS FIBRILLAR STRUCTURES

Within the past quarter-century, numerous students of protozoology have reported fibrillar structures in many flagellate and ciliate Protozoa. Morphological observations were soon followed by various suggestions concerning the functions of fibrils. Although several heterotrichous ciliates have been studied by various investigators in their researches on fibrils, nothing seems to be known of the fibrillar structures of Blepharisma which is easily obtained and maintained in laboratory cultures. Therefore a cytological study of a species of this genus, with particular reference to its fibrillar structures, was undertaken.

The species under observation has been cultured in the Protozoological Laboratory of the University of Illinois for many years. It possesses characteristics common to members of the genus Blepharisma. Its primary specific characters include: separation of the macronucleus into 3 - 6 medium-sized beads; presence of red pigment; mesosaprobic habitat in fresh water; body length, 125 - 165 microns; body width, 0.28 - 0.32 body length; peristome length, 0.4 body length; and rounded posterior end. These characteristics most closely approximate those of *Blepharisma persicinum* Perty, 1852. For the present, the species used in this investigation is held to be *Blepharisma persicinum.*

The Feulgen nucleal reaction often demonstrates in macronuclear beads small vacuolations, spherules, and granules similar to those described by Young in 1939 in the early vegetative stage of the macronucleus in *Blepharisma undulans.* The macronuclear beads in conjugants of *B. persicinum* become vacuolated, and an outer row of vacuoles and an inner honey-combed area can be distinguished after Schaudinn's fixation and Heidenhain's haematoxylin. This appearance coincides with the structure of the macronuclear bulbs in *B. undulans* at the time the central bulb is absorbed by the cytoplasm and the terminal bulbs coalesce. Occasionally in *B. persicinum* can be found two to four large granular nuclear bodies, each containing a small eccentric achromatic vacuole within which is a spherical chromatic body. Such structures correspond to the macronuclear primordia of *B. undul-*

ans which develop from the syncaryon and contain the transforming micronuclei. These observations indicate that the macronucleus of *B. persicinum* is probably reorganized in a manner somewhat similar to that of *B. undulans.*

The ectoplasm is corrugated longitudinally with alternate ridges and valleys. The ridges are filled with red granules which are somewhat regularly arranged in frequently interrupted rows. The granules contain the pigment, zoopurpurin, which may be bleached if the organisms are placed in strong light, which was also shown by Giese in 1938 for *Blepharisma undulans.* Such granules have been interpreted by Stolte in 1924 as trichocysts, by Klein in 1930 as protrichocysts, by Lucas in 1934 as fatty granules, and by Horning in 1927 as mitochondria. They collect at the cell surface in the manner of fatty substances. Owing to their reactions with fixing agents and their similarity to fatty substances described in other protozoans as chondriosomes, it is suggested that the ectoplasmic granules are analogous to chondriosomes of other Protozoa and the Metazoa.

With dark field illumination of living organisms, a canal can sometimes be seen extending from the contractile vacuole to the exterior, which has also been observed in *Blepharisma undulans* by Moore in 1934. Golgi material such as Moore described in *B. undulans* has not been observed in *B. persicinum.*

The wet silver method developed by Gelei and Horvath in 1931 was used with modifications to demonstrate fibrils in *Blepharisma persicinum.* Unchlorinated tap water was substituted for distilled water when washing after fixation; the organisms were bathed in silver nitrate for 30 minutes or longer; and silver reduction occurred in unchlorinated tap water in direct sunlight. Internal fibrils were demonstrated by fixation with Yocom's picro-mercuric mixture and staining with acid fuchsin and light green.

The fibrillar system of *Blepharisma persicinum* consists of the following fibrils and associated structures:

1. An UNDULATING MEMBRANE composed of a row of fine cilia fused in life, bordering the right side of the peristome, extending from the anterior end of the peristome to the left side of the cytopharynx, and folding over the cytostome; and at its base, an UNDULATING MEMBRANE STRAND composed of a row of basal

granules set so closely together that they form a basal strand.

2. A band of MEMBRANELLES beginning at the anterior end of the peristome, proceeding along the left border (peristomal membranelles), passing through the cytostome and spiralling about the wall of the cytopharnyx in a complete circle (pharyngeal membranelles), each membranelle composed of two parallel rows of fused cilia; and at the base of each membranelle, two parallel rows of basal granules forming the BASAL PLATE OF THE MEMBRANELLE.

3. An INNER and an OUTER MEMBRANELLE FIBRIL connecting the inner and outer ends of the series of membranelle plates, joining at the apex of the peristome, and meeting the undulating membrane strand.

4. A PHARYNGEAL COMPLEX consisting of (A) a funnel-shaped CYTOPHARYNX composed of a solid sheath of highly differentiated ectoplasm, (B) PHARYNGEAL MEMBRANELLES and very coarse PHARYNGEAL MEMBRANELLE FIBRILS which give rise to (C) four to six OESOPHAGEAL FIBRILS extending into the endoplasm and usually deflected to the dorsal side.

5. The LONGITUDINAL CILIARY FIBRILS arising from the outer membranelle fibril and the undulating membrane strand and proceeding in a somewhat spiral course over the surface of the body to the cytopygal area.

6. A row of BASAL GRANULES adjacent to, but not connected with each longitudinal ciliary fibril, and from which arise the BODY CILIA.

The components of the fibrillar system can be observed in the living organism with comparative ease, although dark field illumination is necessary to distinguish the oesophageal fibrils.

Four movements are used by *Blepharisma persicinum* in locomotion:

1. Swimming or forward movement with rotation in a clockwise direction (as seen when the animal travels away from the observer) performed by the membranelles and undulating membrane.

2. Forward movement without rotation while on a surface, also brought about by the membranelles and undulating membrane.

3. An avoiding reaction or movement backward, accomplished

by the body cilia while the membranelles are at rest and the undulating membrane spreads out to maintain balance.

4. Turning about a center, brought about by the anterior half of the membranelles resting, the posterior half beating rapidly, and the undulating membrane spreading out as a balance.

Penard's observations in 1922 on the feeding habits of *Blepharisma persicinum* were confirmed. The undulating membrane folds over the cytostome after ingestion of a particle to prevent its exit. Selectivity is exercised to a high degree in choosing food.

The membranelles create a current bringing in food from the outside; sweep the food into the cytopharynx; in the cytopharynx create a vortex sucking food into the lower portion for formation of food vacuoles; are the principal means of forward locomotion; and make possible turning in a circle by keeping one portion stationary and rapidly beating the other.

The undulating membrane forms a barrier to prevent food from being swept across the peristome; aids in sweeping food into the cytopharynx; covers the cytostome to prevent loss of food; and in reverse and circling movements, maintains the equilibrium of the organism.

Contraction of the anterior portion of the body was observed, as when in contact with an object or bending the anterior portion slightly upon entering an unfavorable environment.

An axial gradient was established along the longitudinal axis of *Blepharisma persicinum* by means of 0.01% potassium cyanide. The succession of reactions to cyanide include backward locomotion and expulsion of undigested food particles upon introduction into cyanide; narcotization of the peristomal membranelles; cessation of the cilia about the cytopyge; disintegration of the peristome beginning at the apex and continuing to the cytopharynx; progressive cessation of the body cilia, beginning in the posterior region and proceeding towards the pharyngeal region; cessation of the pharyngeal membranelles and pharyngeal body cilia. The metabolic rate is greatest in the apical region, least in the pharyngeal region, and reaches a secondary maximum in the posterior region.

The fibrils of *B. persicinum* may possess four functions: Coordination, contraction, support, and transfer of metabolic influ-

ences. The primary function is conduction of impulses and coordination of organelles, evidence for which is based on four grounds: (1) Morphological Relationships: Size, position, and general appearance of fibrils, their connection with all movable portions of the body, coordination of the parts thus connected, and activities involving four basic movements and selectivity of food which require coordination that would be most suitably achieved through such a system, all point toward a coordinating function for fibrils in Blepharisma. (2) Chemical Reactions: Both the fibrils of Blepharisma and the nerve fibers of metazoans have affinity for acid fuchsin and silver. (3) Axial Gradient: Based on cessation of ciliary action, the anterio-posterior gradient shows the morphological anterior end to be the physiological anterior end, is similar to gradients of Metazoa which possess conductile systems organized along a longitudinal axis, and is the type expected if the fibrillar system is conductile. (4) Comparison with Metazoan Nervous Tissue: In addition to the affinity of both protozoan fibrils and metazoan nerve fibers for acid fuchsin and silver, their origin and formation is in the outermost layer of the body, ectoplasm in Protozoa and ectoderm in Metazoa.

Contraction and support are secondary functions. The longitudinal ciliary fibrils probably serve for contraction of the anterior portion of the body. The pharyngeal membranelle fibrils are much coarser than the peristomal membranelle fibrils, probably strengthening and maintaining the shape of the cytopharynx. It has been suggested that fibrils may transfer metabolic influences due to proximity of fibrils to the nucleus, as the oesophageal fibrils of *Blepharisma persicinum* which terminate near the macronucleus on the dorsal side of the body. Possession of a moniliform macronucleus makes this function improbable but not impossible.

Lack of connection between the basal granules and the longitudinal ciliary fibrils does not destroy the concept of coordination, for the granules and fibrils are embedded in protoplasm which has the fundamental property of conductivity. Thereby a continuous path exists for transmission of nervous impulses. Sensory and motor impulses are probably carried by the same fibril. The fibrillar system of Blepharisma represents a primitive

type of nervous system, less complex than systems found in the more complicated ciliates. Until micro-surgical techniques are applied to determine the exact functions of the fibrils in Blepharisma, the term "fibrillar system", a purely morphological designation, is used in preference to "neuromotor system", a physiological designation, or "silver line system", a micro-technical designation.

The fibrils in *Blepharisma persicinum* are impregnated by virtue of their sublimatophily and argentophily. Distilled water removes the mercuric chloride after fixation and the silver chloride during reduction. The presence of these chlorides in the fibrils is necessary for silver impregnation, and therefore undistilled natural water containing chlorides is substituted for distilled water to prevent removal of the chlorides by the water.

SUMMARY

1. Through observations on the living organism and by means of wet silver preparations according to the technique of Gelei and Horvath in 1931, there was demonstrated in a heterotrichous ciliate, *Blepharisma persicinum*, a fibrillar system consisting of (a) an undulating membrane strand and an undulating membrane, (b) a band of membranelle plates and membranelles, (c) an inner and an outer membranelle fibril, (d) a pharyngeal complex composed of the cytopharynx, two pharyngeal membranelle fibrils, membranelles, and oesophageal fibrils, (e) longitudinal ciliary fibrils and cilia, and (f) the basal granules of cilia, membranelles, and the undulating membrane.

2. The fibrillar system functions primarily in coordination of the locomotor organelles and ingestatory activity, possesses secondary functions of contraction and support, and possesses possibly a metabolic function. The function of coordination is demonstrated by (a) morphological relationships, (b) chemical reaction of the fibrils to acid fuchsin and silver, (c) establishment of an anterio-posterior gradient of the type to be expected if the fibrillar system is conductile, and (d) comparison with metazoan nervous tissue.

3. Activities of the living organism are analyzed and four movements are described and explained: (a) swimming, (b) forward movement without rotation, (c) an avoiding reaction (back-

ward movement), and (d) a circling movement.

4. The fibrillar system of *Blepharisma persicinum* is interpreted as representative of a primitive or simple system in comparison with more complicated systems of coordination found in the more complex ciliates.

5. The presence of chlorides is believed necessary for appearance of the fibrillar picture in the wet silver technique. Unchlorinated tap water is substituted in the process for distilled water, since the latter removes the necessary chlorides. The technique is explained on the basis of simple chemical reactions.

6. The species of Blepharisma utilized in this investigation is described and found to be synonymous with *Blepharisma persicinum* Perty, 1852.

7. The general morphology of the species is described. Three stages in the reorganization of the macronucleus are described and compared with similar stages found in *Blepharisma undulans* by Young in 1939. Fatty granules filling the ridges of the ectoplasm are suggested to be analogous to chondriosomes. The existence of a canal leading from the contractile vacuole to the exterior is partially confirmed upon the basis of dark field observations.

VITA

Charles Leplie Kanatzar was born in St. Elmo, Illinois, on April 12, 1914. He attended the elementary school in St. Elmo, Illinois, and graduated from the St. Elmo High School in 1931. From 1931 to 1935, he attended the Eastern Illinois State Teachers College, Charleston, Illinois, from which he received in 1935 the degree, Bachelor of Education, with honors. From 1935 to 1939, he was engaged in graduate study at the University of Illinois, receiving the degree, Master of Science, in August, 1936. The summers of 1937 and 1938 were spent in graduate study and research at the Chesapeake Biological Laboratory, Solomons Island, Maryland.

During the academic years of 1933-1934 and 1934-1935, he served as laboratory assistant in zoology at the Eastern Illinois State Teachers College. From 1935 to 1938, he was employed as an assistant in zoology while pursuing graduate study.

He is a member of Kappa Delta Pi, Sigma Xi, Phi Sigma, Gamma Alpha, and the Illinois State Academy of Science.